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ANTIDEPRESANT FLUOXETINE NORMALIZES BRAIN JNKS SIGNALING IMPAIRED BY CHRONIC STRESS IN FEMALE BUT NOT IN MALE WISTAR RATS

M. Mitić, I. Simić, M. Radojčić, M. Adžić

Department of Molecular Biology and Endocrinology, Institute of Nuclear Science Vinča, University of Belgrade
P.O. Box 522, 11001 Belgrade, Serbia

Abstract
c-Jun N-terminal kinases (JNKs) are important stress-responsive kinases that act as mediators in synaptic remodeling and neuronal degeneration in response to stress and, thus receiving considerable attention as potential therapeutic targets. In the present study, we investigated how exposure to chronic stress (chronic psychosocial isolation, CPSI) and subsequent therapy with antidepressant fluoxetine (FLU) affects the nuclear JNKs signalling and its phosphorylation status in the hippocampus (HIPPO) of the female and male Wistar rats. Our results showed that CPSI disrupted nuclear JNKs signaling in a gender specific way, while concomitant FLU treatment normalized JNKs only in HIPPO of females.

Introduction
Increasing evidence implicates stress as an important factor in the vulnerability to depressive and other mental illnesses [1]. Pathways mediating the cellular responses to various stressors include activation of c-Jun N-terminal kinases (JNKs), a subgroup of mitogen-activated protein kinases (MAPKs), that are considered as central transducers in the mammalian brain [2]. Indeed, reduced nuclear levels of JNKs were found in depressed patients and connected with different neuropathological events which are considered to be important in pathophysiology of depression [3]. The JNKs are encoded by at least three genes (JNK1, -2, and -3) that encode 46 kDa (JNK1) and two 54 kDa JNK isoforms (JNK2 and 3), respectively which are activated after phosphorylation [4]. Upon activation in the cytosol JNKs are translocated to the nucleus where they phosphorylate different transcription factors, resulting in enhanced or inhibited genes expression [5]. In the present study, we investigated nuclear total JNKs (tJNKs) levels and its phosphorylation status, pJNKs, in hippocampus (HIPPO) of female and male Wistar rats exposed to chronic psychosocial isolation stress (CPSI) and concomitantly treated with the antidepressant fluoxetine (FLU).

Experimental
Adult female and male Wistar rats were divided into four groups (each consisted of n=10): (I) Control, (II) Control+FLU, (III) CPSI (IV) CPSI+FLU. The experiment consisted of two phases and lasted for 6 weeks (42 days). The first phase (CPSI)

lasted 21 days, during which animals of CPSI and CPSI+FLU were exposed to CPSI. The second phase consisted of treatment with vehicle (VEH) or FLU for the next 21 day. The CPSI animals remained isolated during treatment. FLU was dissolved in water and administered intraperitoneally (5mg/kg body mass) at daily base between 9:00 a.m. and 9.30 a.m. 24 hours after receiving the final dose animals were sacrificed, their hippocampi were removed and used for preparation of the nuclear fraction and the molecular analyses.

The proteins of interest, total, and phospho JNKs were detected using Western blot with β-actin as a loading control. Data are presented as a mean ± SEM and in each gender were analyzed with two-way ANOVA. To determine statistically significant gender differences we used three-way ANOVA employing stress, fluoxetine and gender as the factors. All statistically significant differences are given as $p < 0.05$.

Results and Discussion

Our results showed that in females CPSI significantly decreased all three total JNKs ($F=13.5$, $F=11.8$, $p<0.05$) (Figure 1a and b) and their phospho-isoforms (pJNKs $F=8.7$, $F=7.5$, $p<0.05$) (Figure 1c and 1d), indicating reduced JNKs signaling under chronic stress. The concomitant FLU treatment significantly increased both tJNK and pJNK levels reversing them to the control levels (tJNKs, $F=21.5$, $F=13.1$, $p<0.05$; pJNKs $F=6.6$, $F=12.6$, $p<0.05$) (Figure 1). In males, CPSI exerted the opposite effect and significantly increased all three nuclear pJNKs ($F=16.1$, $F=14.5$, $p<0.05$), not affecting tJNKs levels, suggesting enhanced signaling of the JNK pathway. Concomitant FLU treatment of CPSI males did not exert statistically significant effect on any of analyzed JNKs, although it slightly increased the levels of both tJNKs and pJNKs. Our findings in females were in accordance with literature, showing decreased levels of nuclear JNKs in depressed patients and their concomitant alteration by antidepressant treatment [6]. Moreover, three-way ANOVA revealed significant gender differences upon CPSI and concomitant FLU treatment regarding the nuclear total and phospho JNKs levels (tJNK, gender x CSPI, $F=7.6$, $p<0.05$,gender x CPSI x FLU interaction, $F=16.4$, $p<0.05$; pJNK gender x CSPI $F=15.4$, $p<0.05$; gender x CSPI x FLU interaction $F=10.2$, $p<0.05$). Namely, in females, CPSI decreased nuclear total and phospho JNKs that was reversed with FLU treatment, while in males CPSI exerted the opposite effect and increased JNKs phosphorylation, and in addition, the effect of FLU was not detected.

Conclusion

CPSI disrupted nuclear JNKs signaling in a gender specific way, while concomitant FLU treatment normalized it in females, but not in males. Namely, in females, CPSI reduced JNKs signaling by decreasing both, total and phospho, nuclear JNKs level, while in males CPSI increased JNKs signaling via its phosphorylation. FLU treatment normalized JNKs signaling in females via its influence on the level of JNKs protein, while in males FLU did not target this signaling pathway.

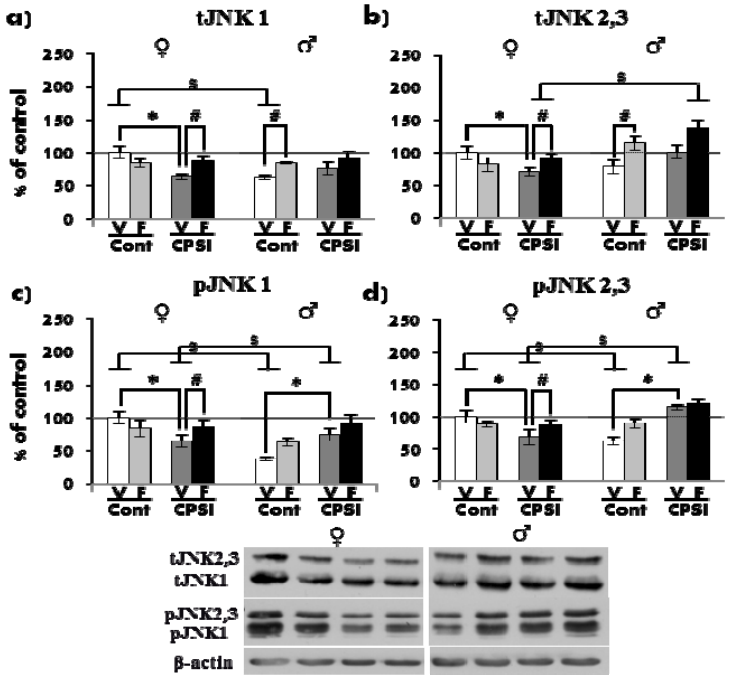

Figure 1. The levels of nuclear total JNKs (tJNK1,2,3) and phospho JNKs (pJNK1,2,3) protein levels (a,b,c,d) in the hippocampus of control (Cont) and stressed (CPSI) female (♀) and male (♂) Wistar rats treated with vehicle (V)/fluoxetine (F). Data are presented as mean ± SEM (**vs.* CPSI, # *vs.* FLU treatment, $ female *vs.* male).

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